Available online at www.sciencedirect.com
SCIENCE DIRECT®
Bioorganic & Medicinal Chemistry Letters 15 (2005) 5197–5201
Bioorganic & Medicinal Chemistry Letters

3-(5-chloro-2,4-dihydroxyphenyl)-Pyrazole-4-carboxamides as inhibitors of the Hsp90 molecular chaperone

Paul A. Brough,[a,*] Xavier Barril,[a] Mandy Beswick,[a] Brian W. Dymock,[a] Martin J. Drysdale,[a] Lisa Wright,[a] Kate Grant,[a] Andrew Massey,[a] Allan Surgenor[a] and Paul Workman[b]

[a]*Vernalis Ltd, Granta Park, Great Abington, Cambridge CB1 6GB, UK*
[b]*Cancer Research UK Centre for Cancer Therapeutics, The Institute of Cancer Research, 15 Cotswold Road, Sutton, Surrey SM2 5NG, UK*

Received 30 June 2005; revised 9 August 2005; accepted 22 August 2005

Abstract—Information from X-ray crystal structures of Hsp90 inhibitors bound to the human Hsp90 molecular chaperone was used to assist in the design of 3-(5-chloro-2,4-dihydroxyphenyl)-pyrazole-4-carboxamides as novel inhibitors of Hsp90. Accessing an extra interaction with the protein via Phe138 gave a significant increase in binding potency compared to similar analogues that do not make this interaction.

Molecular chaperones are proteins which have a key role in the maintenance of conformation, stability, and function of client proteins within the cell. Heat shock protein 90 (Hsp90) is an ATP-dependent molecular chaperone that has several oncogenic client proteins involved in signal transduction, cell cycle regulation, and apoptosis, and has recently become a focus of interest as a potential anticancer drug target.[1–6]

It has been shown that several natural products and their derivatives have anti-tumor activity arising from inhibition of the intrinsic ATPase activity of Hsp90[7–10] (Fig. 1). Inhibition of Hsp90 activity results in the proteasomal degradation of client proteins causing cell growth arrest and/or apoptosis in cancer cells.[11]

The geldanamycin derived inhibitor 17-AAG (**1b**) has entered phase II clinical trials and initial results are encouraging, providing proof of principle for Hsp90 inhibitors as cancer therapeutics.[12–14] However, 17-AAG has several potential limitations including poor solubility, limited bioavailability, toxicity, and extensive metabolism.[15] These issues and the inherent chemical complexity of the compounds have led to significant efforts to identify small molecule inhibitors of Hsp90.[16–21] Figure 2 shows some of the chemotypes recently identified as potent inhibitors of Hsp90.[18,22,19]

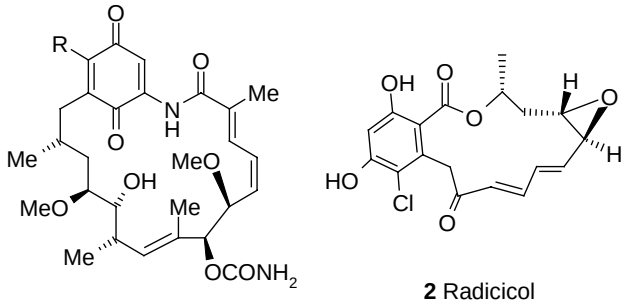


Figure 1. Structures of natural product derived Hsp90 inhibitors.

We have disclosed previously how structure-based drug design was implemented in the identification of **5** (VER-49009) using structural information from the X-ray crystal structure of **3** (CCT018159) bound to the ATPase

Keywords: Hsp90; Cancer; Structure-based drug design; Pyrazole; X-ray crystallography.
* Corresponding author. Tel.: +44 1223 895555; fax: +44 1223 895556; e-mail: p.brough@vernalis.com

0960-894X/$ - see front matter © 2005 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2005.08.091

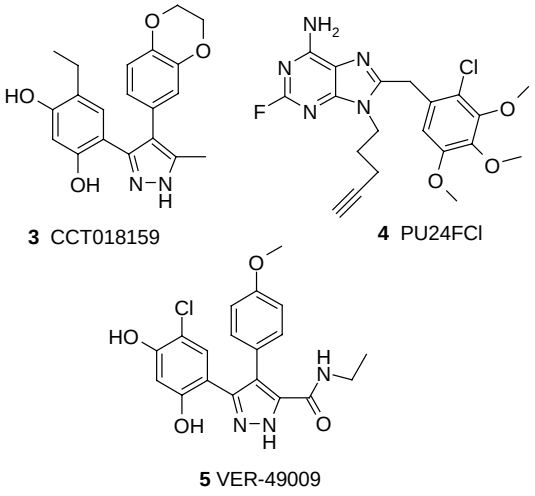
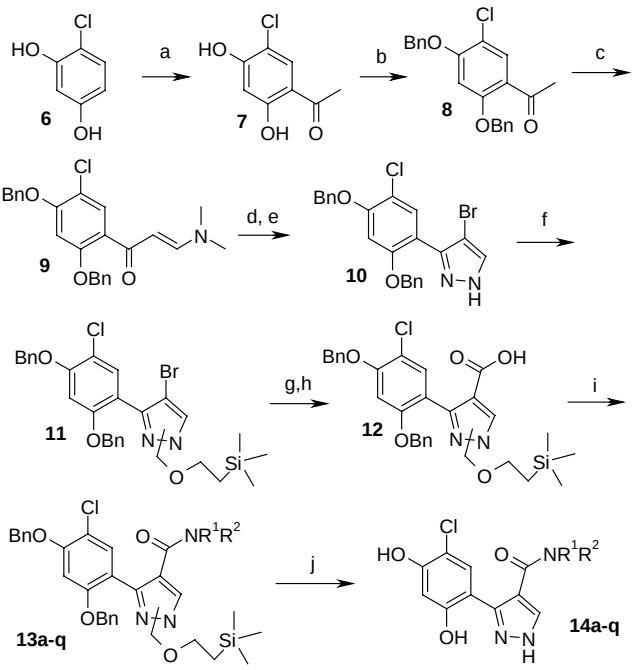


Figure 2. Small molecule inhibitors of Hsp90.

binding site of Hsp90α.[19,20] This information enabled us to successfully target a specific region of the protein to design a significantly more potent analogue. Herein, we report a series of novel 3-(5-chloro-2,4-dihydroxyphenyl)-pyrazole-4-carboxamides[23] designed with a similar approach as that used to identify **5**. In the present case however, we have targeted additional interactions at a distinct region of the Hsp90 protein.

Scheme 1 depicts the synthesis of carboxamides **14a–q**: Commercially available 4-chlororesorcinol **6** underwent regioselective Friedel–Crafts acylation followed by benzyl protection to give the acetophenone **8**. Generation of the requisite pyrazole ring was achieved via reaction of **8** with DMF.DMA and reaction of the resulting eneamine **9** with hydrazine hydrate. Bromination of the pyrazole was regioselective for the 4-position affording compound **10**. After some detailed investigation of protecting groups, the pyrazole nitrogen was protected as a silyl ethoxy methoxy (SEM) ether, affording **11** as a 1:1 mixture of regioisomers, (reflecting the expected existence of tautomeric forms of the pyrazole). Lithium–halogen exchange with **11** at −78 °C afforded the 4-lithio species which was subsequently quenched with carbon dioxide to give carboxylic acid **12**. A standard HATU mediated coupling reaction was then performed with a series of amines to generate amides **13a–q**. Treatment of amides **13a–q** with boron trichloride effected concomitant removal of the benzyl and SEM protecting groups affording pyrazole carboxamides **14a–q** (Table 1).

N-terminal human Hsp90α his-tagged protein was crystallized in complex with compounds **14h** and **14o** as previously described.[24] Data were collected on both co-crystals and the structures were subsequently solved by molecular replacement using the previously solved native Hsp90α structure[24] (PDB code: 1UY1). Both crystals diffracted in space group P21 to resolutions of 1.9 and 1.7 Å for **14h** and **14o**, respectively. Co-crystals of Hsp90 with **14o** bound were grown at a slightly different pH (pH 5.5) as compared to those containing **14h** (pH 6.5). In both structures, the flexible loop region adopts a more 'open' conformation as previously

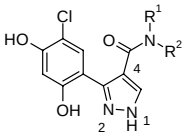


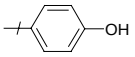
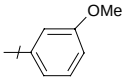
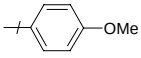
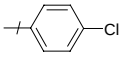
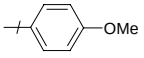
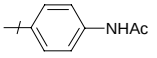
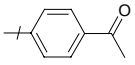
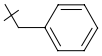
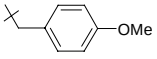
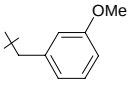
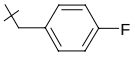
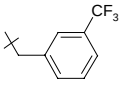
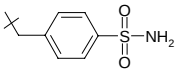
Scheme 1. Synthesis of 3-(5-chloro-2,4-dihydroxyphenyl)-pyrazole carboxamides. Reagents and conditions: (a) $AcOH{\cdot}BF_3$, 90 °C, 3.5 h, 58%; (b) BnBr, K_2CO_3, MeCN, reflux, 6 h, 90%; (c) DMF·DMA, 150 °C, 2 h, 84%; (d) $NH_2NH_2{\cdot}H_2O$, EtOH, reflux, 5 h, 92%; (e) NBS, CH_2Cl_2, rt, 2 h, 97%; (f) SEMCl, Cs_2CO_3, DMF, rt, 16 h, 90%; (g) *n*-BuLi, THF, −78 °C, 15 min, 45%; (h) CO_2, −78 °C to rt; (i) R^1R^2NH, HATU, DIPEA, DMF, 100 °C, 10 min; (j) BCl_3, CH_2Cl_2, rt, 16 h.

described.[25] The binding mode of the resorcinol and pyrazole rings is essentially as described before,[19,20,26] The novel and most prominent features of the structure are described in the next section.

Structural information gained from X-ray crystallography of Hsp90 inhibitors (including **3**) bound to human Hsp90α protein revealed that the 4-position of the pyrazole could be elaborated to give further interaction with the Hsp90 protein and potentially increase both binding potency and activity against cells via inhibition of intracellular Hsp90. Using the available structural information, phenyl and benzyl templates were initially evaluated for generation of SAR in this region of the molecule. All compounds were tested for binding at the ATP binding site using a fluorescence polarization assay,[27] with the majority of these being additionally tested for their ability to inhibit the growth of HCT116 human colon cancer cells in vitro. The data in Table 1 show the effects of modification to the templates. Most of the compounds discussed inhibit binding of the probe substrate in the low micromolar range, notable exceptions being the tertiary amide **14f** (which is >50 μM compared to its secondary amide analogue **14d**) and heteroaryl compounds **14p** and **14q** which also lose an appreciable amount of binding potency. However, incorporation of an acetyl at the *para* position of the aryl template (compound **14h**) gives a significant increase in binding potency. The crystal structure of **14h** in complex with the N-terminus domain of Hsp90α reveals that the ketone moiety is hydrogen bonded to

Table 1. Binding and growth inhibition assay results for compounds **14a–14q**



Compound	R^1	R^2	FP enzyme IC_{50}, μM[a]	Cell growth inhibition (GI_{50}), μM
14a	H		7.1	nd
14b	H		14.7	>80
14c	H		11.7	>80
14d	H		6.0	>80
14e	H		6.9	>80
14f	Me		>50	nd
14g	H		31.5	59.7
14h	H		**0.258**	**11.6**
14i	H		12.8	>80
14j	H		6.0	60.7
14k	H		10.7	>80
14l	H		5.35	56.2
14m	H		5.4	45.0
14o	H		0.461	>80
14p	H		35.6	nd
14q	H		>50	nd

[a] Values are means of two experiments. nd, not determined.

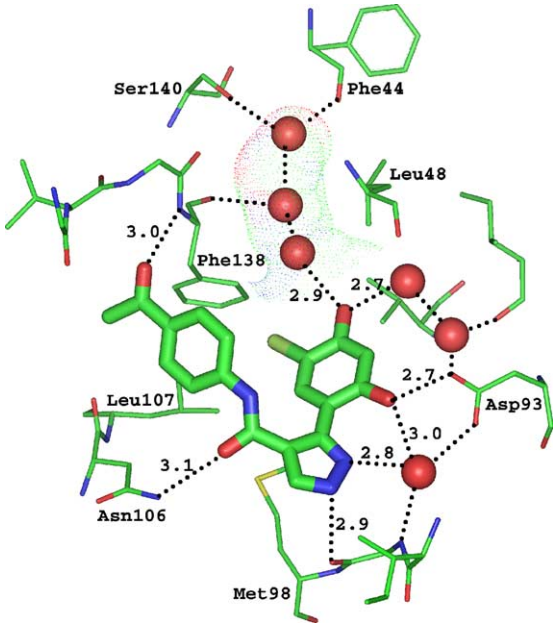


Figure 3. Binding mode of **14h** to Hsp90. The dotted surface renders the new cavity formed between helices 2 and 7.

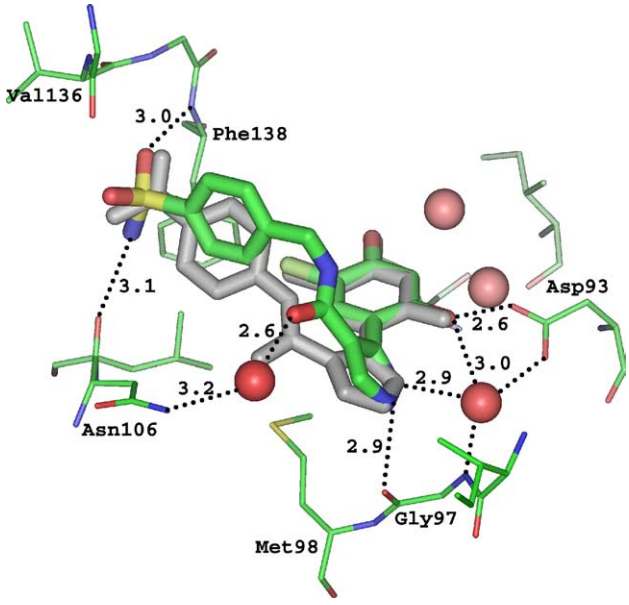


Figure 4. Binding mode of **14o** to Hsp90. Compound **14h** is depicted in silver for comparison.

the backbone of Phe138, thus explaining the higher affinity for Hsp90 (Fig. 3). It is worth noting that the α-phosphate of ADP[28] and the amidic oxygen of geldanamycin[25] are also hydrogen bonded to Phe138 but, to the best of our knowledge, this is the first time that the impact of this interaction can be quantified.

The benzylic compound **14o** also exhibits increased binding affinity for Hsp90 and crystallography again identified a hydrogen bond between Phe138 and the ligand as responsible (Fig. 4), though this compound does not display any cellular activity in vitro (possibly due to poor cell penetration inherent with primary sulfonamides). The intermolecular hydrogen bond with Phe138 increases the binding affinity of compounds **14h** and **14o** by more than one order of magnitude compared to similar compounds unable to place a hydrogen bond acceptor at this position. This equates to approximately 1.5–2.0 kcal/mol, clearly in the upper range of the contribution of hydrogen bonds to protein stability[29–31] or protein–ligand binding,[32–34] which is surprising for such a solvent-exposed site.

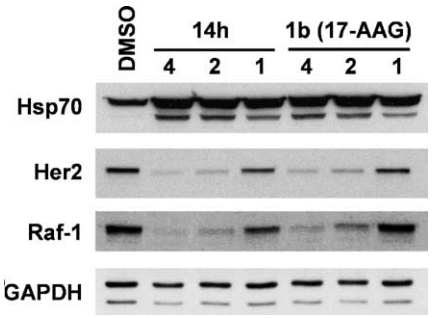


Figure 5. Western blot showing increases in Hsp70 and decrease in Her2 and Raf-1 following exposure of HCT116 cells to **14h** and **1b**.

The crystal structure of **14h** also reveals a new channel created at the interface between α-helices 2 and 7, at the bottom of the ATP binding pocket. This is the result of a displacement of the side chain of Leu48, which is relatively minor but sufficient to leave space for a chain of 3 water molecules hydrogen bonded to the ligand and the protein (Fig. 3). This channel has never been reported before, although we have noted that the PDB structure 1YC3[26] presents an occluded water molecule at a position corresponding to the bottom of the channel. The implications of this channel for ligand recognition or biological function of Hsp90 are unclear, but its potential exploitability for ligand binding should receive further consideration.

To support the premise that the disclosed effects on cell growth were via Hsp90 inhibition, Western blotting was used to study the effect of **14h** and 17-AAG (**1b**) on in vitro cellular levels of Hsp70, Her2, Raf-1, and CDK4 in HCT116 cells (GAPDH used as a loading control). Figure 5 shows that **14h** causes induction of Hsp70 (at 1, 2, and 4 times GI_{50}) and knock down of Raf-1 and Her2 (at 2 and 4 times GI_{50}). CDK4 gave partial knock down at 8 times GI_{50} (data not shown). The observed changes are consistent with the known molecular signature of Hsp90 inhibition.[35,36]

In conclusion, we have utilized structure-based drug design to assist in the design and rationalization of Hsp90 inhibitors. The backbone of residue Phe138 has been identified as an additional interaction point to increase binding potency. This structural information may be useful to help design new inhibitors of the Hsp90 chaperone.

Crystallographic coordinates have been deposited with the Protein Data Bank: [**14h** PDB code: 2BYH; **14o** PDB code: 2BYI]

Supplementary Information

Full experimental procedures for the synthesis of **14h** are available. In addition a protocol for the Fluorescent Polarization assay is provided.

References and notes

1. Jolly, C.; Morimoto, R. I. *J. Natl. Cancer Inst.* **2000**, *92*, 1564.
2. Workman, P. *Curr. Cancer Drug Targets* **2003**, *3*, 297.
3. Neckers, L.; Ivy, S. P. *Curr. Opin. Oncol.* **2003**, *15*, 419.
4. Solit, D. B.; Scher, H. I.; Rosen, N. *Semin. Oncol.* **2003**, *30*, 709.
5. Whitesell, L.; Bagatell, R.; Falsey, R. *Curr. Cancer Drug Targets* **2003**, *3*, 349.
6. Kelland, L. R.; Sharp, S. Y.; Rogers, P. M.; Myers, T. G.; Workman, P. *J. Natl. Cancer Inst.* **1999**, *91*, 1940.
7. Schulte, T. W.; Neckers, L. M. *Cancer Chemother. Pharmacol.* **1998**, *42*, 273.
8. Schulte, T. W.; Akinaga, S.; Soga, S.; Sullivan, W.; Stensgard, B.; Toft, D.; Neckers, L. M. *Cell Stress. Chaperones* **1998**, *3*, 100.
9. Neckers, L.; Schulte, T. W.; Mimnaugh, E. *Invest. New Drugs* **1999**, *17*, 361.
10. Kaur, G.; Belotti, D.; Burger, A. M.; Fisher-Nielson, K.; Borsotti, P.; Riccardi, E.; Thillainathan, J.; Hollingshead, M.; Sausville, E. A.; Giavazzi, R. *Clin. Cancer Res.* **2004**, *10*, 4813.
11. Hostein, I.; Robertson, D.; DiStefano, F.; Workman, P.; Clarke, P. A. *Cancer Res.* **2001**, *15*, 4003.
12. Banerji, U.; O'Donnell, A.; Scurr, M.; Pacey, S.; Stapleton, S.; Asad, Y.; Simmons, L.; Maloney, A.; Raynaud, F.; Campbell, M.; Walton, M.; Lakhani, S.; Kaye, S.; Workman, P.; Judson, I. *J. Clin. Oncol.* **2005**, *23*, 4152.
13. Goetz, M. P.; Toft, D.; Reid, J.; Ames, M. M.; Stensgard, B.; Safgren, S.; Sloan, J.; Atherton, P.; Vasile, V.; Salazaar, S.; Adjei, A.; Croghan, G.; Erlichman, C. *J. Clin. Oncol.* **2005**, *23*, 1078.
14. Grem, L.; Morrison, G.; Guo, X.-D.; Agnew, E.; Takimoto, C. H.; Thomas, R.; Szabo, E.; Growchow, L.; Grollman, F.; Hamilton, J. M.; Neckers, L.; Wilson, R. H. *J. Clin. Oncol.* **2005**, 1885.
15. Workman, P. *Cancer Lett.* **2004**, *206*, 149.
16. Dymock, B. W.; Drysdale, M.; McDonald, E.; Workman, P. *Expt. Opin. Ther. Patents* **2004**, *14*, 837.
17. Kasibhatla, S. R.; Boehm, M. F.; Hong, K. D.; Biamonte, M. A.; Shi, J.; Le Brazidec, J.-Y.; Zhang, L.; Hurst, D. PCT Int. Appl., W0 2005/028434, **2005**.
18. Vilenchik, M.; Solit, D.; Basso, A.; Huezo, H.; Lucas, B.; He, H.; Rosen, N.; Spampinato, C.; Modrich, P.; Chiosis, G. *Chem. Biol.* **2004**, *11*, 787.
19. Cheung, K.-M. J.; Matthews, T. P.; James, K.; Rowlands, M. J.; Boxall, K. J.; Sharp, S. Y.; Maloney, A.; Roe, S. M.; Prodromou, C.; Pearl, L.; Aherne, W.; McDonald, E.; Workman, P. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 3338.
20. Dymock, B. W.; Barril, X.; Brough, P. A.; Cansfield, J. E.; Massey, A.; McDonald, E.; Hubbard, R. E.; Surgenor, A.; Roughley, S.; Webb, P.; Workman, P.; Wright, L.; Drysdale, M. *J. Med. Chem.* **2005**, *48*, 4212.
21. Llauger, L.; He, H.; Kim, J.; Aguirre, J.; Rosen, N.; Peters, U.; Davies, P.; Chiosis, G. *J. Med. Chem.* **2005**, *48*, 2892.
22. Dymock, B.; Barril, X.; Beswick, M.; Collier, A.; Davies, N.; Drysdale, M.; Fink, A.; Fromont, C.; Hubbard, R. E.; Massey, A.; Surgenor, A.; Wright, L. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 325.
23. Beswick, M. C.; Brough, P. A.; Drysdale, M. J.; Dymock, B. W., PCT Int Appl. WO 2004/050087, **2004**.
24. Wright, L.; Barril, X.; Dymock, B.; Sheridan, L.; Surgenor, A.; Beswick, M.; Drysdale, M.; Collier, A.; Massey, A.; Davies, N.; Fink, A.; Fromont, C.; Aherne, W.; Boxall, K.; Sharp, S.; Workman, P.; Hubbard, R. E. *Chem. Biol.* **2004**, *11*, 775.
25. Stebbins, C. E.; Russo, A. A.; Schneider, C.; Rosen, N.; Hartl, F. U.; Pavletich, N. P. *Cell* **1997**, *89*, 239.
26. Kreusch, A.; Han, S.; Brinker, A.; Zhou, V.; Choi, H. S.; He, Y.; Lesley, S. A.; Caldwell, J.; Gu, X. *J. Bioorg. Med. Chem. Lett.* **2005**, *15*, 1475.
27. A protocol for the Fluorescent Polarization assay is provided in the Supplementary Information Section. In

addition, this information is also available within reference 23.
28. Obermann, W. M.; Sondermann, H.; Russo, A. A.; Pavletich, N. P.; Hartl, F. U. *J. Cell Biol.* **1998**, *143*, 901.
29. Shirley, B. A.; Stanssens, P.; Hahn, U.; Pace, C. N. *Biochemistry* **1992**, *31*, 725.
30. Chen, Y. W.; Fersht, A. R.; Henrick, K. *J. Mol. Biol.* **1993**, *234*, 1158.
31. Takano, K.; Yamagata, Y.; Funahashi, J.; Hioki, Y.; Kuramitsu, S.; Yutani, K. *Biochemistry* **1999**, *38*, 12698.
32. Fersht, A. R.; Shi, J. P.; Knill-Jones, J.; Lowe, D. M.; Wilkinson, A. J.; Blow, D. M.; Brick, P.; Carter, P.; Waye, M. M.; Winter, G. *Nature* **1985**, *314*, 235.
33. Connelly, P. R.; Aldape, R. A.; Bruzzese, F. J.; Chambers, S. P.; Fitzgibbon, M. J.; Fleming, M. A.; Itoh, S.; Livingston, D. J.; Navia, M. A.; Thomson, J. A. *Proc. Natl. Acad. Sci. U.S.A.* **1994**, *91*, 1964.
34. Le, T. I.; Freitag, S.; Klumb, L. A.; Chu, V.; Stayton, P. S.; Stenkamp, R. E. *Acta Crystallogr. D. Biol. Crystallogr.* **2003**, *59*, 1567.
35. Maloney, A.; Workman, P. *Expert Opin. Biol. Ther.* **2002**, *2*, 3.
36. Clarke, P. A.; Hostein, I.; Banerji, U.; Stefano, F. D.; Maloney, A.; Walton, M.; Judson, I.; Workman, P. *Oncogene* **2000**, *19*, 4125.